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Abstract: Tissue oxygen saturation (StO2) is a valuable clinical parameter e.g. for intensive care applications or monitoring during surgery. Studies showed that near-infrared spectroscopy (NIRS) based tissue oximeters of different brands give systematically different readings of StO2. Usually these readings are linearly correlated and therefore StO2 readings from one instrument can easily be converted to those of another instrument. However, it is interesting to understand why there is this difference. One reason may be that different brands employ different spectra of hemoglobin. The aim here was to investigate how these different absorption spectra of hemoglobin affect the StO2 readings. Therefore, we performed changes in StO2 in a phantom experiment with real human hemoglobin at three different concentrations (26.5, 45 and 70 M): desaturation by yeast consuming the oxygen and re-saturation by bubbling oxygen gas. The partial pressure of O2 in the liquid changed from at least 10 kPa to 0 kPa and ISS OxiplexTS, a frequency-domain NIRS instrument, was used to monitor changes of StO2. When we employed two different absorption spectra for hemoglobin, StO2 values were comparable in the normal physiological range. However, particularly at high and low StO2 values, a difference of >6% between these two spectra were noticed. Such a difference of >6% is substantial and relevant for medical applications. This may partly explain why different brands of NIRS instruments provide different StO2 readings. The hemoglobin spectra are therefore a factor to be considered for future developments and applications of NIRS oximeters.

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Hemoglobin spectra affect measurement of tissue oxygen saturation

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ABSTRACT

Tissue oxygen saturation (StO_2) is a valuable clinical parameter e.g. for intensive care applications or monitoring during surgery. Studies showed that near-infrared spectroscopy (NIRS) based tissue oximeters of different brands give systematically different readings of StO_2. Usually these readings are linearly correlated and therefore StO_2 readings from one instrument can easily be converted to those of another instrument. However, it is interesting to understand why there is this difference. One reason may be that different brands employ different spectra of hemoglobin. The aim here was to investigate how these different absorption spectra of hemoglobin affect the StO_2 readings. Therefore, we performed changes in StO_2 in a phantom experiment with real human hemoglobin at three different concentrations (26.5, 45 and 70 µM): desaturation by yeast consuming the oxygen and re-saturation by bubbling oxygen gas. The partial pressure of O_2 in the liquid changed from at least 10 kPa to ~0 kPa and ISS OxiplexTS, a frequency-domain NIRS instrument, was used to monitor changes of StO_2. When we employed two different absorption spectra for hemoglobin, StO_2 values were comparable in the normal physiological range. However, particularly at high and low StO_2 values, a difference of >6 % between these two spectra were noticed. Such a difference of >6 % is substantial and relevant for medical applications. This may partly explain why different brands of NIRS instruments provide different StO_2 readings. The hemoglobin spectra are therefore a factor to be considered for future developments and applications of NIRS oximeters.

Keywords: Hemoglobin spectra, tissue oxygen saturation, near-infrared spectroscopy

1. INTRODUCTION

Non-invasive and continuous bedside measurement of tissue oxygen saturation (StO_2) by near-infrared spectroscopy (NIRS) is relevant for many clinical applications like (neonatal) intensive care or monitoring during surgery.

Studies has shown that different brands of NIRS oximeters provide systematically different StO_2 values[1, 2]. This does not inhibit the clinical application of NIRS, because in general the StO_2 values of different NIRS instrument are linearly correlated with high correlation coefficients [1, 2]. It is therefore possible for a clinician and other users to compare own values with values obtained from a different instrument in the literature. However, there is a need to determine what the origin of this difference is.

One difference compared to other parameters is that the StO_2 cannot be measured by any other method except NIRS and there is no gold standard available to compare to. Currently, work is under way to define an ISO norm, but it seems that it will be difficult to define a procedure how to determine a gold standard StO_2. In addition, most manufacturers did not publish their algorithms how to calculate StO_2 from raw optical data. Although this impedes a factual understanding of the origin of these different StO_2 readings it is possible to take some reasonable guesses about the signal processing steps of

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these algorithms. One fundamental part of such an algorithm must be the absorption spectra of chromophores of interest, i.e. oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb). These spectra are needed to transform the measured light intensities to StO_2 values. Several such spectra have been measured and published, but are not completely congruent even though they are similar.

The aim of this paper was to determine whether such slight deviations would lead to significant differences in StO_2 readings for an instrument with a published algorithm.

2. METHODS

2.1 Instrument

In this study, we employed the commercially available OxiplexTS (ISS, Champaign, Illinois, USA) frequency-domain NIRS device. This device was chosen because all raw data is completely accessible and the algorithm to calculate the StO_2 has been published [3-5]. The OxiplexTS has two light sources (692 nm and 834 nm) whose intensities are modulated with 110 MHz. The light is transmitted to the tissue and the back reflected light is collected by optical fibers. At the detector, the radiofrequency is heterodynely demodulated by modulating the high voltage of the photomultiplier tube at 110.005 MHz. The rigid sensor of the OxiplexTS implements a multi-distance geometry with four source-detector separations of 2.5, 3.0, 3.5 and 4.0 cm. For each distance, the mean intensity, amplitude and phase is measured. From the change in the phase and amplitude with distance, the absolute values for the tissue absorption (μ_a) and reduced scattering coefficients are calculated [3].

2.2 Algorithm to calculate StO_2

From the measured μ_a values at the two wavelengths, the absolute O_2Hb and HHb concentration values were calculated according to.

$$\mu_a = \alpha \times c \,, \tag{1}$$

with α the molecular absorption coefficient and c the concentration of the chromophore. In our case, we have a 2 x 2 system of equations for two wavelengths and two chromophores (O_2Hb and HHb) (equation 2). In addition, since the tissue generally contains a lot of water, this is taken into account by estimating the water concentration (in %) of the medium under investigation and calculating the contribution of the water to μ_a ($\mu_{a,water}$) [5].

$$\begin{pmatrix} \mu_{a,692nm} - \mu_{a.water,692nm} \\ \mu_{a,834nm} - \mu_{a.water,834nm} \end{pmatrix} = \begin{pmatrix} \alpha_{O2Hb,692nm} & \alpha_{HHb,692nm} \\ \alpha_{O2Hb,834nm} & \alpha_{HHb,834nm} \end{pmatrix} \times \begin{pmatrix} c_{O2Hb} \\ c_{HHb} \end{pmatrix}. \tag{2}$$

Equation 2 can be solved for the concentrations by

$$\begin{pmatrix} c_{O2Hb} \\ c_{HHb} \end{pmatrix} = \begin{pmatrix} \alpha_{O2Hb,692nm} & \alpha_{HHb,692nm} \\ \alpha_{O2Hb,834nm} & \alpha_{HHb,834nm} \end{pmatrix}^{-1} \times \begin{pmatrix} \mu_{a,692nm} - \mu_{a.water,692nm} \\ \mu_{a,834nm} - \mu_{a.water,834nm} \end{pmatrix}. \tag{3}$$

For this study we used the standard absorption coefficients of water for the ISS instrument [6]. For the hemoglobin spectra, we used the ones by Wray [7] and Prahl [8]. The values we included are displayed in Table 1.

Table 1. Molecular absorption coefficients from Wray [7] and Prahl [8].

Wavelength	O_2Hb in (1/(cm*mM))		HHb in (1/(cm*mM))	
	Wray	Prahl	Wray	Prahl
692 nm	0.9556	0.6392	4.5821	4.6063
834 nm	2.3671	2.2832	1.7891	1.5949

2.3 Phantom setup

The phantom setup is described in detail by Kleiser et al. [1] and was intended to simulate the head of a preterm infant, a prime application for NIRS. It consisted of a container with a window made of a layer of silicone with optical properties and thickness (2.5 mm) similar to the scalp and skull of a typical neonate. The container was constantly stirred by a magnetic stirrer and heated to keep a temperature of approximately 37°.

The rigid sensor of the OxiplexTS was placed on the window and fixed with a clamp specifically constructed for this purpose. In addition, a partial pressure of oxygen (pO_2) sensor (Microx TX trace3, NTH-PSt1, PreSens - Precision Sensing GmbH, Regensburg, Germany) was immersed in the phantom.

The container was first filled with a liquid mainly containing human hemoglobin and Intralipid®, which was added to obtain the desired scattering coefficient of 5.5 cm^{-1}. In addition, we added buffer to keep the pH at physiological levels. The measurement with the OxiplexTS was started and detector voltage was automatically adjusted. After a few minutes. yeast was added to deoxygenate the hemoglobin. To increase the speed of deoxygenation glucose was added to feed the yeast. Once the pO_2 stabilized at 0 kPa, we started bubbling O_2 gas until the pO_2 was >10 kPa.

The total hemoglobin concentration [tHb] was initially set to 26.5 µM and after two desaturations it was raised in to 45 µM. After a further desaturation, it was increased to 70 µM. This is a range that is typical for neonatal brain.

2.4 Data processing

Raw mean values for intensity, amplitude and phase were stored in a computer and absorption and scattering coefficients were calculated according to the ISS algorithm. StO_2 was calculated for the Wray and Prahl spectra separately in a special script of Matlab R2014a (Mathworks, Inc, Mass., USA) according to the equations above.

3. RESULTS

In Fig. 1 the whole time trace of the experiment is depicted. The StO_2 is displayed for both Wray (in black) and Prahl (in grey) spectra in the top graph. The tHb concentration is indicated in the upper part of the figure. The middle graph displays the pO_2 values and the bottom graph the difference between the StO_2 calculated using Wray spectra and the one for Prahl spectra.

It is visible that the pO_2 linearly decreased initially and once the StO_2 started to decrease, the slope of the pO_2 decrease flattened. The relation between the StO_2 and pO_2 corresponds to the well-known sigmoidal-shaped dissociation curve which is strongly non-linear. The precise shape depends on several factors such as temperature, pH, and pCO_2. Therefore, it is not straightforward to predict StO_2 from pO_2. It can however be assumed that at 0 kPa the StO_2 is 0% and at pO_2 > 10 kPa the StO_2 is close to 100%.

At ~90min and ~170 min additional hemoglobin was added. Since this additional hemoglobin contains oxygen, its injection leads to a small increase in pO_2 and StO_2. It is visible that the noise level increased when the tHb concentration increased. This is due to the lower number of photons that reach the detector and hence an increased contribution of shot noise. During the last desaturation, there are some outliers visible. These occur when the detector voltage is adjusted to reach a higher dynamic range of the instrument. This is needed to cope with the large changes in absorption that are much higher than usual physiological changes. The difference in StO_2 between Wray and Prahl spectra (Fig. 1 bottom) changes with the oxygenation of the hemoglobin. At high StO_2 it reaches 6.5 % and at low StO_2 it corresponds to -11.2 %. In the normal

physiological range between 55 % and 75 % StO_2, this difference is much smaller (between 0.02 % and 3.08 % at StO_2 range corresponding to Wray's spectra between 55.2% to 75.26 %).

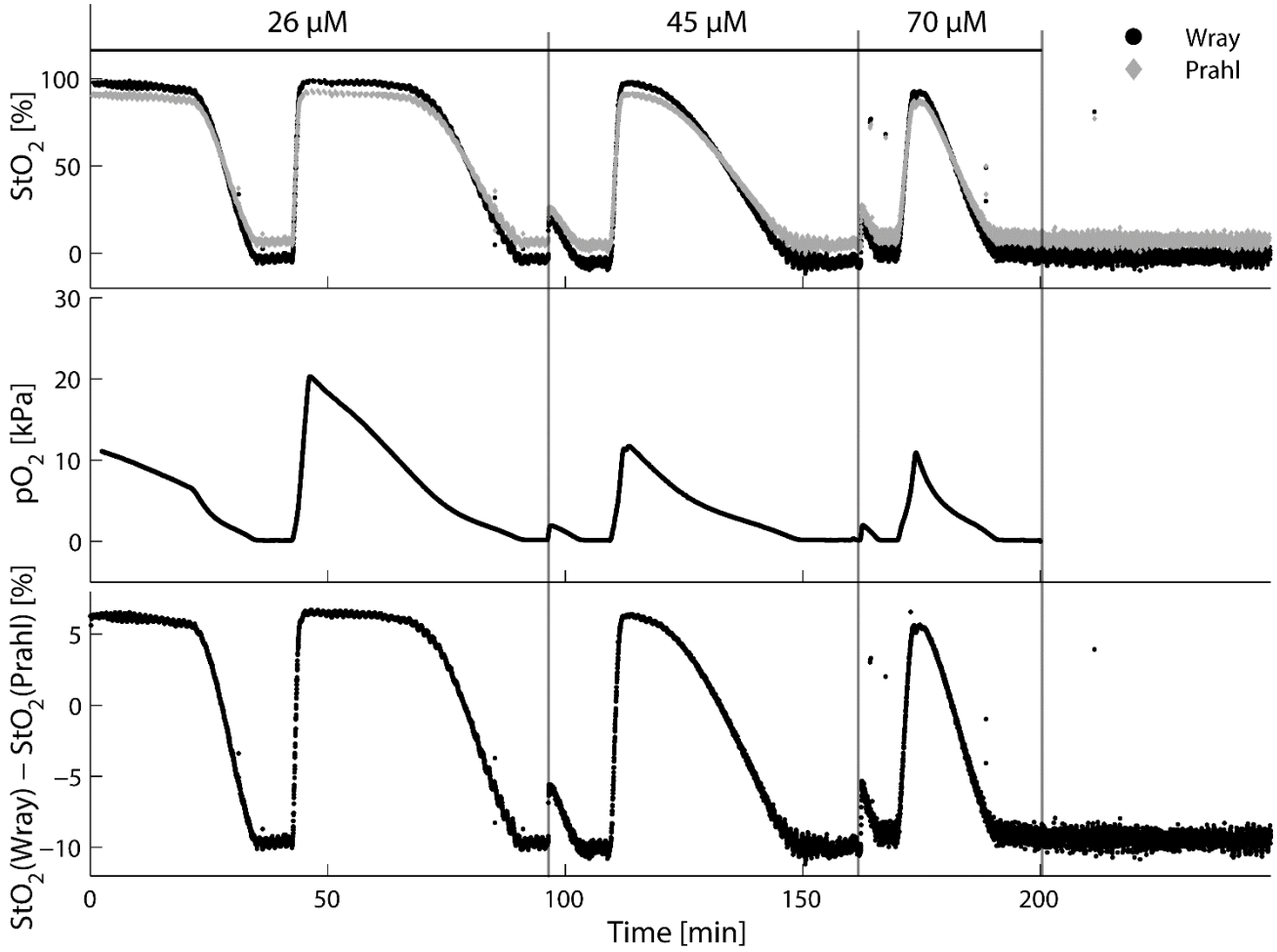

Figure 1. This figure visualizes the time traces of the StO_2 using the Wray [7] and Prahl [8] spectra (top panel), the pO_2 (middle panel), and the difference between StO_2 according to Wray and the StO_2 according to Prahl.

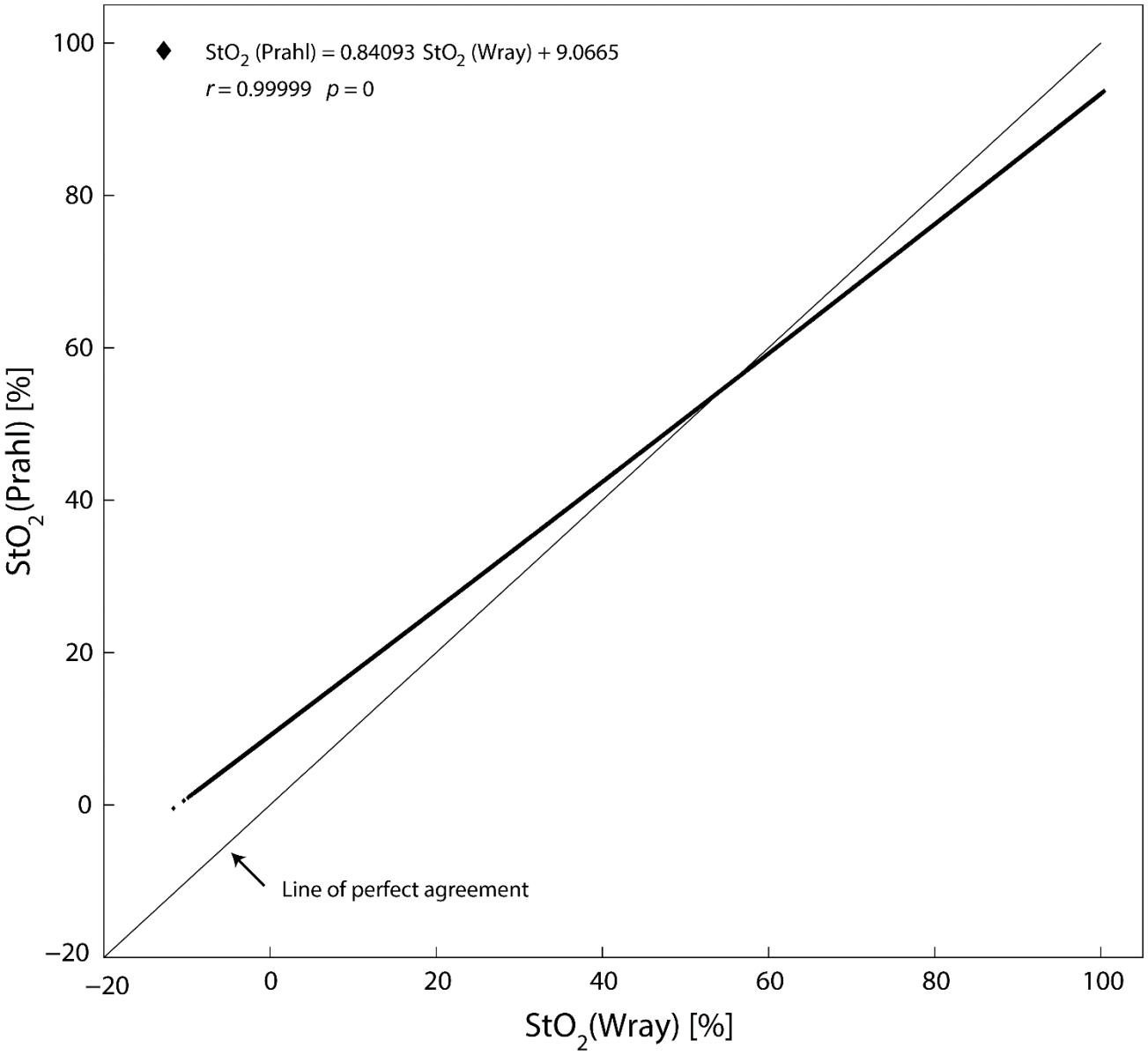

Figure 2. This figure visualizes the linear correlation between the StO_2 using the Wray [7] on the x-axis and the Prahl [8] spectra on the y-axis. Due to the nature of the 2 x 2 matrix to calculate the two StO_2s, it is natural that this linear correlation occurs. The slope and intercept are displayed in the inset.

4. DISCUSSION

It is well-known that the O_2Hb and HHb spectra slightly vary between different publications. It is important to be aware that these small differences (Table 1) lead to substantial differences in the absolute StO_2 values (Fig. 1). This demonstrates that indeed the molecular absorption spectrum of hemoglobin matters and may account for the systematic differences in StO_2 found between the different brands of NIRS instruments.

We would like to point out the following limitations of the study: The detected differences in StO_2 depend on the wavelengths that a NIRS instrument employs. The values displayed in this study are true for the OxiplexTS instrument. For other instruments, this difference may be smaller or larger, but it is likely that this effect is present there as well.

It would be interesting to investigate this effect further. Why are the molecular absorption spectra different between publications? Which of the spectra is more reliable? Maybe further measurements of hemoglobin spectra with a higher accuracy are necessary?

While the Wray spectra were recorded with human blood samples, the Prahl spectra are a mixture of data from two sources and no information is provided how the data was obtained exactly. There are other spectra available [9].

In our case, we would like to suggest that the Wray [7] spectra, that are employed for the OxiplexTS instrument are more accurate than the Prahl [8] spectra, because the StO_2 (Wray) reaches 100 % StO_2 at $pO_2 > 10$ kPa and is closer to 0 % StO_2 at $pO_2 = 0$ kPa which correspond to the expected values, while the StO_2 (Prahl) only reaches 93.5 % StO_2 at $pO_2 > 10$ kPa and 6.8 % StO_2 at $pO_2 = 0$ kPa. Also in a recent study[9] to determine the accuracy of the measurement of [HbO_2], [Hb] and [tHb], the spectra of Wray resulted in more accurate values compared to the spectra of Prahl.

5. CONCLUSION

When we employ two different absorption spectra for hemoglobin, in the normal physiological range StO_2 values were comparable. However, in particular at high and low pO_2, there is a substantial and relevant difference in StO_2 of >6% between these two spectra. Such a difference >6% is substantial and relevant and may partly explain, why different brands of NIRS instruments provide different StO_2 readings. Thus, the hemoglobin spectra are a factor that needs to be considered for future developments and applications of NIRS oximetry.

6. ACKNOWLEDGEMENTS

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